

THE SUBTRIBAL AFFINITIES OF THE GENUS *TETRAGONOTHECA*
(ASTERACEAE: HELIANTHEAE)

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ABSTRACT

Tetragonotheca is a North American herbaceous genus of four species traditionally placed in the tribe Heliantheae, subtribe Helianthinae, and more recently placed in the subtribe Galinsoginae. The melampolide type sesquiterpene lactones reported in *Tetragonotheca* strongly suggest that *Smallanthus* of the Melampodiinae is its closest generic ally. A comparison of morphological features of representative species offers further support for this concept.

KEY WORDS: Systematics, Asteraceae, Heliantheae, *Tetragonotheca*, North America.

Tetragonotheca as treated by Turner & Dawson (1980) comprises 4 species: *T. helianthoides* which grows throughout much of the coastal plain of the southeastern United States, and *T. ludoviciana*, *T. repanda* and *T. texana* which are endemic to or primarily occur in central or southeastern Texas. The genus is a member of the Heliantheae, but has questionable subtribal affinities. Stuessy (1977) placed *Tetragonotheca* in Helianthinae along with genera such as *Encelia* Adans., *Flourensia* DC., *Helianthus* L., *Viguiera* Kunth and yet others. Turner & Dawson (1980) noted *Tetragonotheca* to be relatively remote from any extant genus of the Helianthinae, but if it had a less specialized, multiseriolate involucre, it would most likely be positioned near *Viguiera*. Robinson (1981) considered *Tetragonotheca* to be a member of subtribe Galinsoginae. Seaman (1982), after laboratory collaboration with the present authors prior to his seminal review of the sesquiterpenes of the Asteraceae, placed *Tetragonotheca* in the subtribe Melampodiinae.

All species of *Tetragonotheca* possess melampolide type sesquiterpene lactones (Figure 1) which are typical of subtribe Melampodiinae, (sensu Stuessy 1977) and these are rarely found elsewhere in the family (Quijano, *et al.* 1979; 1980; Seaman, *et al.* 1979; 1980; Seaman & Fischer 1980; Seaman 1982). Most of the Helianthinae (sensu Stuessy 1977) examined to date, other than

Tetragonotheca, have distinctive lactones of the heliangolide and germacrolide types (Figures 3 & 4). The Galinsoginae (sensu Robinson 1981) are not known to possess lactones. These observations suggest that the subtribal placement of *Tetragonotheca* is open to question. This study reviews the lactone data and examines the morphological and cytological data relative to the classification of *Tetragonotheca*.

Methods and Materials

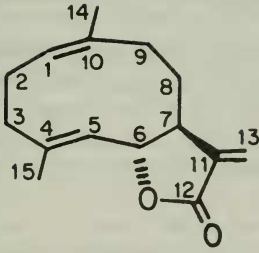
The sesquiterpene lactones of *Tetragonotheca* are reported in a series of phytochemical papers (Quijano, *et al.* 1979; 1980; Seaman & Fischer 1980; Seaman *et al.* 1979; 1980; Seaman 1982). Morphological comparisons are based on field observations, specimens preserved in FAA, herbarium specimens and data from appropriate literature. Photographs of the achenes were taken with a Wild dissecting microscope equipped with a Nikon camera. Scanning electron microscopy procedures are those given in Urbatsch & Wussow (1979) and Wussow & Urbatsch (1979).

Results and Discussion

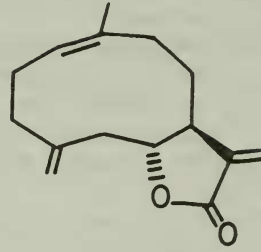
Chemical Evidence. Twenty-six sesquiterpene lactones have been isolated and characterized from *Tetragonotheca* (Quijano, *et al.* 1979; 1980; Seaman, *et al.* 1979; Seaman & Fischer 1980). The compounds are of two skeletal types, melampolides and repandolides (Figures 1 & 2). The melampolides are characterized by having a cis-, trans-cyclodecadiene skeleton; repandolides are biosynthetically modified melampolides (Seaman *et al.* 1979; Fischer *et al.* 1979).

Tetragonotheca helianthoides and *T. ludoviciana* contain only melampolides that have the same 10-membered ring skeleton but differ in their ester side chains (Quijano, *et al.* 1979; 1980; Seaman & Fischer 1980). Fifteen lactones, the highest number for the genus, have been isolated from *T. ludoviciana* (Quijano, *et al.* 1979; 1980). Fourteen of the lactones are unique to *T. ludoviciana* and the remaining, polydalin, has been previously isolated from *Smilax uvealensis* (Herz & Bhat 1970). Twelve of these 14 unique compounds have five-carbon ester side chains at C-8 and C-9 with hydroxyls, or their oxidative derivatives, at C-2' or C-3'; the two remaining lactones possess five-carbon esters at position C-8 but have acetates at position C-9 (Quijano, *et al.* 1979; 1980).

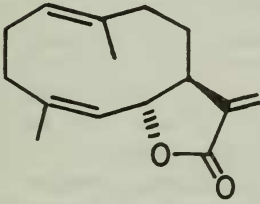
Six melampolide type lactones were detected in *T. helianthoides*, (Seaman & Fischer 1980; Seaman, *et al.* 1980). Four of these have the same skeletal type as the melampolides in *T. ludoviciana*. Of these four compounds, two contain five-carbon ester side chains at C-8 and acetates at C-9, and the other two have this same configuration with hydroxyl groups at C-15. The two remaining melampolides of this species have five-carbon esters at C-8 and C-9.



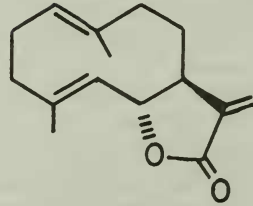
1. melampolide



2. repandolide



3. heliangolide



4. germacrolide

Figures 1-4. Types of germacranolides found in the Heliantheae. 1. melampolide. 2. heliangolide. 3. germacrolide. 4. repandolide.

Tetragonotheca repanda contains only repandolides with five-carbon ester functions at C-4 and C-8 and five-carbon ester moieties at C-9 (Quijano, *et al.* 1979). Based on thin-layer chromatographic data, *T. texana* contains the same four lactones.

The skeletal types of sesquiterpene lactones in *Tetragonotheca* are identical to those found in several genera belonging to Melampodiinae (Seaman, *et al.* 1980; Seaman & Fischer 1980). Polydalin, from *T. ludoviciana*, and longipilin, from *T. repanda*, were, in fact, first isolated from *Smallanthus uvedalius* and *Melampodium longipes* B.L. Robins., respectively (Herz & Bhat 1970; Fischer, *et al.* 1979).

The occurrence of 6,12-lactonized melampolides outside the Melampodiinae is limited to species of *Tetragonotheca*, *Enhydra fluctuans* Lour., *Blainvillea dichotoma* (Murr.) Cass. (Seaman *et al.* 1980; Seaman 1982; Bohlmann, *et al.* 1981; 1982), *Milleria quinqueflora* L. (Jakupovic, *et al.* 1987) all of the tribe Heliantheae. *Urospermum dalechampii* Schmidt (Rychlewska, *et al.* 1986) and *Lactuca sativa* L. both of the tribe Lactuceae (Mahmoud, *et al.* 1986) also possess 6,12-lactonized melampolides.

That *Tetragonotheca* is best placed in the Melampodiinae on the basis of lactone data was suggested by Urbatsch (1979) and Seaman, *et al.* (1980) where it was subsequently placed by Seaman (1982). The position of *Enhydra* Lour. remains controversial. Benthams (1873) includes it in the Melampodiinae, Stuessy (1977) transfers it to the Ecliptinae Less. and Robinson (1981) places it in the monogeneric Enhydrinae H. Robinson. Seaman, *et al.* (1980) notes that *Enhydra* might fit well in the Melampodiinae, but deferred a decision until data from more than one species becomes available. The skeletal types of lactones found in one species of *Blainvillea* Cass. are similar to those in *Tetragonotheca*, but the two differ in the types of sidechains; aldehyde substituent groups characterize the former and carbomethoxy groups characterize the latter and the Melampodiinae (Bohlmann, *et al.* 1981; Fischer, *et al.* 1979; Seaman 1982). Stuessy (1977) and Robinson (1981) regard *Blainvillea* to be in Ecliptinae, even though its lactone chemistry is quite different from that known for the subtribe. Further investigation of its disposition is suggested by the chemical data.

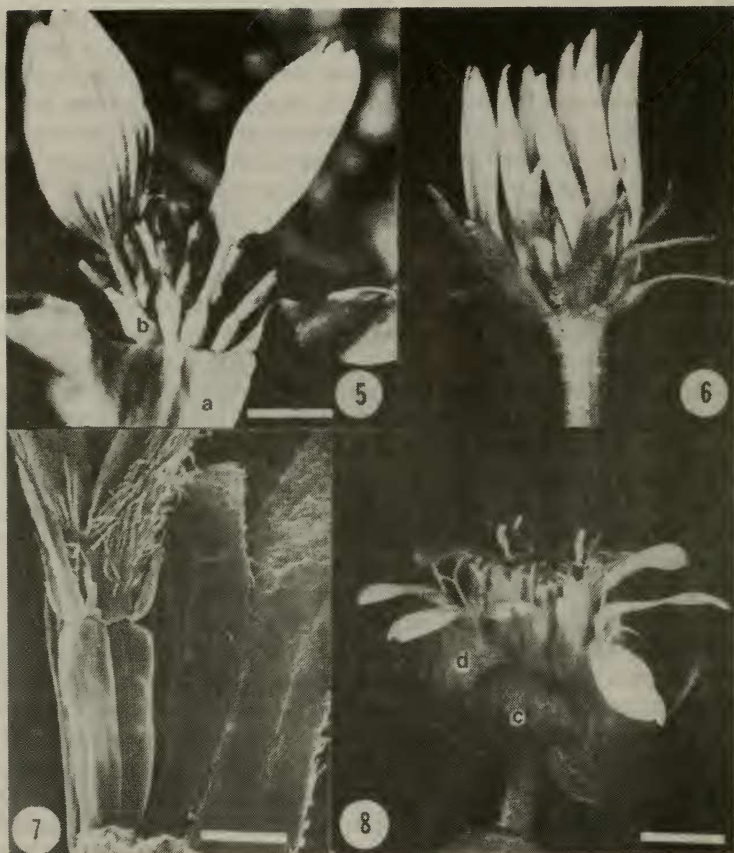
The occurrence of melampolides in *Milleria quinqueflora* of subtribe Milleriinae Benth. & Hook. supports its close phyletic position with the Melampodiinae (Jakupovic, *et al.* 1987). Except for *Milleria*, there is complete disagreement on the generic composition of the Milleriinae as proposed by Stuessy (1977) and Robinson (1981), the two most recent investigators of the group. Stuessy places three of the six genera of Robinson's (1981) Milleriinae in the Melampodiinae and Robinson places one genus of Stuessy's Melampodiinae in the Milleriinae.

Melampolides from *Lactuca* and *Urospermum* (subtribe Lactuceae) are very similar to those found in the Melampodiinae/Milleriinae complex, but differ in not being substituted at C-8 (Mahmoud, *et al.* 1986; Rychlewska, *et al.* 1986). Their presence in such remotely related taxa (tribe Lactuceae) is undoubtedly due to convergence and clearly points to the need to use biochemical data in concert with other sorts of information in making systematic judgments.

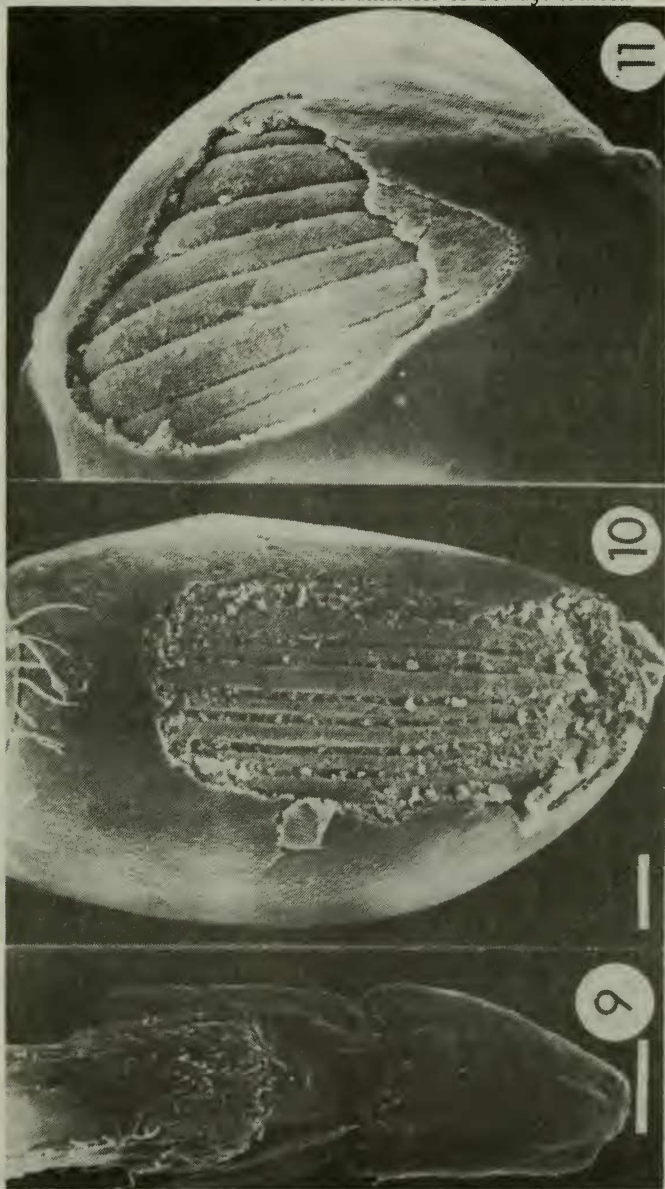
Melampolides lactonized in the 8,12 configuration have been isolated from *Iva frutescens* L. (Herz, *et al.* 1972) and *Schkuhria schkuhrioides* Thell. (Stewart & Mabry 1985), which are treated by Robinson (1981) as members of Ambrosiinae Less. and Chaenactidinae Rydb., respectively. Little is known about the biosynthesis and genetic basis for the various lactones. Nevertheless, differences between compounds of the latter two taxa and the ones producing the 6,12-lactonized melampolides probably have a genetic basis, making them useful phylogenetic markers.

The lactone data tend to reject previous suggested affiliations of *Tetragonotheca* with such genera as *Viguiera*, *Tithonia*, *Encelia* and *Helianthus* (Stuessy 1977), which produce heliangolides and germacrolides, and with genera of the Galinsoginae (Robinson 1981), which are not known to possess lactones. Because the lactone data strongly suggest that *Tetragonotheca* may be allied to the Melampodiinae, we undertook this evaluation of the morphological criteria relative to its classification.

Morphological Evidence. *Tetragonotheca* shares some features with Helianthinae (*sensu* Stuessy 1977) including yellow rays, hermaphroditic disc flowers, and base chromosome numbers of $x = 17$ (Turner & Dawson 1980). However, the majority of morphological features, together with the lactone data, argues for excluding *Tetragonotheca* from the Helianthinae. The leaves and inflorescence bracts of *Tetragonotheca* are mostly opposite (upper leaves and bracts alternate in *T. repanda*) and its petioles encircle the stem, its involucre is biseriate, the outer phyllaries are four in number, broad and herbaceous, the inner series resemble the receptacular bracts, and each closely subtends a ray achene (Figure 5). Helianthinae typically have alternate leaves above and mostly narrow leaf bases, multiseriate involucre and numerous, narrow phyllaries with the inner ones not uniformly corresponding with the rays and not enclosing their achenes (Figures 6 & 7). Rays in the Helianthinae are usually neuter; whereas, *Tetragonotheca* has fertile rays (Figure 5) similar to those found in *Smallanthus* (Figure 8). When present, the pappus of most Helianthinae is often caducous and generally consists of two relatively large awns or scales sometimes with smaller intermediate scales (Figure 9). *Tetragonotheca* usually lacks pappus or when present (such as in *T. ludoviciana*), it consists of numerous, readily deciduous small scales of one size class. Achenes are usually compressed in Helianthinae and prismatic to cylindrical in *Tetragonotheca* (Figures 9 & 10).



Figures 5-8. Morphological features of species in *Tetragonotheca*, *Smallanthus* and *Helianthus*. 5. Flowering head of *T. helianthoides* (Urbatsch 2750 LSU) showing biseriate involucre; "a" large outer phyllaries; "b" inner phyllaries associated with ray florets. Scale markers = 5 mm for figures 5 and 6. 6. Flowering head of *H. simulans* (Urbatsch et al. 2698 LSU) showing multiseriate involucre. 7. Ray flower of *H. simulans* with subtending associated planar phyllary. Scale markers = 1 mm in figures 7 and 8. 8. Flowering head of *S. uvedalius* (Fischer & Wilzer 139 LSU), "c" outer phyllaries; "d" inner phyllaries associated with ray florets.



Figures 9-11. Achenes of *Helianthus*, *Tetragonotheca* and *Smallanthus*.
 9. Ovary of disc flower of *H. simulans* showing somewhat flattened achenes, pappus, and typical corolla base. Scale marker = 1 mm. 10. Immature ray achenes of *T. helianthoides* with part of the fleshy covering removed showing striations underneath. Scale markers = 0.5 mm in figures ten and eleven. 11. Immature ray achenes of *S. uvedalius* showing fleshy covering and striations.

Although *Tetragonotheca* resembles the Galinsoginae (sensu Robinson 1981) in such features as opposite leaves, fertile rays and hermaphroditic disc flowers, these similarities may not offer a strong case for a close relationship. *Tetragonotheca* differs in involuclral and paleae features; its leaves and corolla tubes possess globular trichomes along with uniseriate ones; it has yellow rays; a deciduous pappus or none at all; and a different base chromosome number $x = 17$ vs 4, 8, or 9 (Robinson, *et al.* 1981). The Galinsoginae (sensu Robinson 1981) are not known to produce sesquiterpene lactones. They rarely have clasping leaves and their leaf trichomes usually consist of uniseriate arcuate hairs. They possess coriaceous or sometimes herbaceous outer phyllaries which are usually subimbricate in two to five series and narrowly lanceolate, often trifid paleae that do not enclose the achenes. Rays in the Galinsoginae are white often with cyanic tinges; disc flowers are hermaphroditic; uniseriate hairs are generally present on the corolla tubes; and pappus scales are usually persistent.

Our investigations suggest that *Tetragonotheca* is similar to *Smallanthus* not only in terpene chemistry but also in morphology. *Smallanthus* has been included in Melampodiinae by both early and modern synantherologists (Bentham 1873; Hoffmann 1890; Stuessy 1977; Robinson 1981; Wells 1965). Features generally diagnostic for the subtribe include opposite leaves, biseriate or pluriseriate involuclres composed of broad outer herbaceous phyllaries and paleae-like inner phyllaries partially or completely enclosing the achenes, and fertile rays (Stuessy 1972, 1977; Robinson 1981). The Melampodiinae sensu Robinson (1981) includes only genera with staminate disc flowers. Stuessy (1977) includes some genera in the subtribe, e.g. *Rumfordia* DC. and *Siegesbeckia* L., with hermaphroditic disc flowers.

Species of *Tetragonotheca*, *Smallanthus* and other Melampodiinae have opposite leaves (in *T. repanda* the upper leaves may be alternate) and opposite inflorescence bracts with clasping bases. In *Tetragonotheca* the outer series of the biseriate involuclre consists of four broad herbaceous phyllaries and the inner series is paleae-like. Each inner bract subtends and partially encloses a ray achene, a characteristic also seen in *Smallanthus*. The latter generally has five outer phyllaries that may be arranged in two series (Figure 8). Corolla tubes of the disc flowers in *Tetragonotheca*, *Smallanthus* and many Melampodiinae have spreading, uniseriate hairs and capitate trichomes. Ray achenes in *Smallanthus* and the ray and disc achenes in *Tetragonotheca* are all fertile and epappose (except for *T. repanda* which frequently has a pappus of numerous small scales). Also, achenes of the two genera are virtually identical in possessing a pericarp composed of a fleshy, loosely attached outer layer and a hard, longitudinally grooved inner layer, a condition seen in other genera in the Melampodiinae (Figures 9, 10 & 11).

The hermaphroditic disc flowers of *Tetragonotheca* represent a major difference between it and *Smallanthus* and of many other species in Melampodiinae which traditionally has been characterized as having disc flowers that produce

only pollen. Recent investigators have tended to de-emphasize the diagnostic importance of this character by transferring genera with hermaphroditic disc flowers (e.g. *Rumfordia*, *Aziniphyllum* Benth. and *Siegesbeckia* L.) to the Melampodiinae (Sanders 1977; Stuessy 1977; Turner 1978a; 1978b). As Turner (1978a) suggests, perhaps excessive reliance has been accorded the sterile disc condition because closely related species in *Siegesbeckia* may possess either male fertile or bisexual disc flowers. McVaugh & Anderson (1972) and McVaugh & Laskowski (1972) note that this single character notwithstanding, *Rumfordia*, *Siegesbeckia*, *Polymnia* (including *Smallanthus*) and *Trigonospermum* may form a natural group because of their numerous similarities. Even though Robinson's Melampodiinae (1981) includes only taxa with fertile rays and sterile disc flowers, he too questions the reliability of this feature, and indeed, genera in his Milleriinae possess both conditions. The previously mentioned investigators have characterized the Melampodiinae as having disc flowers with fused style branches. Except for being smaller in size, we have observed the disc flower style branches of *Smallanthus uvedalius* to be divided and similar in form to those in *Tetragonotheca*.

Cytological Evidence. The haploid complement of $n = 17$ for *Tetragonotheca* does not coincide with the proposed base chromosome numbers of $x = 10$ and 15 (Stuessy 1977) and $x = 5$ and 6 (Turner 1978a) for the Melampodiinae. However, the diversity of chromosome numbers reported for *Smallanthus* ($n = 16, 17, 18$ and higher) and additional numbers of $n = 9, 10, 11, 12, 19$, etc. for other Melampodiinae (Stuessy 1977) suggests a possible ancestral aneuploid origin for the *Tetragonotheca* genome.

Tetragonotheca is similar to *Smallanthus* of the Melampodiinae in several chemical and morphological features. The occurrence of the unusual melampolide lactones has supported our question of the association of *Tetragonotheca* with genera of the Helianthinae and the Galinsoginae. Chemical approaches represent a powerful tool in systematic investigations (Bendz & Santesson 1973) especially when used in concert with data from other sources. In this case, chemical data lead to expansion and refinement of our morphological investigations, and suggested a more natural classification for the taxa in question.

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